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RICE CULTIVARS (*Oryza Sativa L*) SUSCEPTIBLE TO IRON TOXICITY HAVE POOR GRAIN NUTRITIONAL QUALITY

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ABSTRACT

Soil iron toxicity is a serious environmental problem of rice crop, grown in acid soils of Eastern India. We investigated the impact of higher iron in the soil on growth, yield and grain quality of three popularly grown rice varieties- Mahsuri, Ranjit and Siyal Sali. Plants of these varieties were grown at control soil, +100 ppm, +200 ppm and +300 ppm levels of Fe^{2+}. Ranjit and Siyal Sali recorded higher shoot Fe concentration with substantial reduction in growth and yield at 300ppm iron compared to Mahsuri. Grain yield was higher in Mahsuri accompanied by better yield attributing parameters at 300 ppm Fe^{2+}. Total soluble protein, starch, and amylose contents in the grains were found to be unaltered in variety Mahsuri. These parameters were found to decline significantly in Ranjit and Siyal Sali at excess Fe^{2+}. A significant impact of higher Fe^{2+} in the growth medium on grain quality of rice is being observed in present investigation. The results show a negative impact of iron toxicity on the nutritional quality of the grains and a genotype tolerant to iron toxicity being also able to maintain good grain quality.

KEYWORDS: Iron Toxicity, Oryza Sativa, Nutrient Imbalance, Grain Quality & Yield

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Original Article

INTRODUCTION

Iron is an essential micronutrient, for the growth and nutrition of rice plants for its Fe (II)/Fe (III) redox system[1]. High concentration of Fe^{2+}in the soil may lead to nutrient imbalance by limiting the absorption of other nutrients[2, 3]. Iron is present in soluble ferrous form in waterlogged soil, via reduction of insoluble Fe^{3+} iron[4, 5]. Excess ferrous iron in plant cause true Fe toxicity by liberating reactive oxygen species (ROS) through Habber-Weishes reactions as well as pseudo iron toxicity by impeding the uptake of other nutrients resulting in nutrient deficiency[6 -- 10]. Depending on the soil condition and cultivar type, the critical concentration causing Fe toxicity can range from, 20 to 2,500 µg Fe per gm leaf dry weights[11].

Severe leaf bronzing or micronutrient imbalance due to higher soil Fe^{2+} are reported to be involved directly or indirectly in the synthesis of leaf amino-acids, proteins, and sugar[2,12--14], which may affect the grain quality components in a rice variety. Information on nutrient transport to developing grains at higher Fe^{2+} concentration in reducible soil environment is inadequate. Higher Fe content in grain of rice is considered as a genetic trait of improved rice varieties[15]. Hence in developing countries, study of grain quality in rice varieties may be a suitable tool for identification of efficient rice cultivars to grow in the soil with iron excess / toxic condition. The present investigation was conducted with the objectives of studying the impact of higher iron on grain yield and grain quality components in few popular rice varieties grown in North Eastern part of India.

MATERIALS AND METHODS

A pot experiment was conducted during monsoon rice season in the year 2014-2015. Soil was collected from a rice field located at Titabor of state Assam, India (soil type-sandy clay loam, total soil iron 345 ppm, pH 5.4, available phosphorus 18.1 kg. ha^{-1}, nitrogen 460 kg. ha^{-1}, potash 127 kg ha^{-1} and organic carbon 1.2%). The experiment was conducted with three rice (Oryza sativa-L) varieties viz. Mahsuri, Ranjit (high yielding varieties) and Siyal Sali (traditional tall variety) and were grown in four different levels of Fe^{2+} in the form of $FeSO_4.7H_2O$. Concentrations of Fe^{2+} solutions added were + 0 ppm (as control), +100 ppm, +200 ppm. and +300 ppm. Treatments were started in the pot one week after transplanting and continued at an interval of seven days up to flowering stage. Treatments were replicated four times in a randomized block design (RBD). Thirty days old seedlings of uniform vigor were transplanted at the rate of three seedlings per pot. A uniform waterlogged environment was maintained, with distilled water in the pots, throughout the experimental period. All the reagents used in the analysis are of analytical grade (Merck GR).

Yield and yield attributing parameters were recorded by standard method[16].

Grain Crude Proteins

The total nitrogen in grain was determined by Micro-Kjeldahl's method[17] with 0.5 g powdered grain. Percentage nitrogen was converted to crude protein by multiplying with a factor 5.95[18].

Total Soluble Protein

Moisture free powdered grain sample (0.5 g) was used to determine total soluble protein spectrophotometrically (Systronics UV-VIS Spectrophotometer 118) at 660 nm[19]. Proteins were fractionated using Osborne's method with some modifications.

Total Soluble Sugar

0.2 g moisture free powdered grain sample was extracted with 80% ethanol, 1ml of extract was treated with anthrone reagent and intensity was measured spectrophotometrically (Systronics UV-VIS Spectrophotometer 118) at 630 nm[20]. The reducible sugar was determined by using the method as described by Miller[21] at 510 nm.

Starch Contents

The sugar free residue was extracted by 52% Perchloric acid. 0.1 ml of the extract was treated with anthrone reagent and starch content was estimated spectrophotometrically at 630 nm[22]. The grain amylose content was determined as described by Sawbhagya and Bhattacharya[23], 0.1 g moisture free powdered grain sample was distilled with ethanol and kept for overnight adding 1N NaOH. The mixture was boiled; 5 ml of the extract was mixed with acetic acid and 0.2% iodine solution. Intensity of the colour developed was measured in spectrophotometer (Systronics UV-VIS Spectrophotometer 118) at 590 nm.

Grain Free Amino Acid

0.1 g of dried material was homogenized and refluxed with 80% ethanol for 15 minutes on a steam bath and centrifuged at 20,000 g for 20 minutes. 0.2 ml of extract was mixed with ninhydrin reagent and absorbance was read in spectrophotometer (Systronics UV-VIS Spectrophotometer 118) at 570 nm[24].

Crude Fiber Content

Crude fiber in the grain was determined by using the method described by Thimmaiah[25].

Shoot Fe at harvest and grain Fe, Cu, Zn and Mn were determined by using Atomic Absorption Spectrophotometer (Chemito, AA 203D)[17]. The K concentration was determined flame photo-metrically[26] while grain P was estimated from mineral solution converting phosphate to phosphomolybdic acid and finally reducing by hydroquinone. The blue colour developed was measured in a spectrophotometer (Systronics UV-VIS Spectrophotometer 118) at 660 nm[26].

Statistical analysis: The experimental design was Randomized Block Design with three varieties, four treatments and four replications. Statistical analyses of experimental data were carried out by using ANOVA (SPSS software). Analysis of variance was carried out to test the significance of treatment effect. F-test, coefficient of variance and critical difference were calculated by standard method[27].

RESULTS AND DISCUSSIONS

Shoot Fe Concentration

All the varieties showed an increasing trend of total Fe concentration in shoots, with the increment of Fe^{2+} than those grown at control soil. Highest shoot Fe concentration was recorded at 300 ppm Fe^{2+}, followed by 200 and 100 ppm Fe^{2+} iron in the growth medium. Among the varieties, a considerable difference in shoot Fe was detected (Figure 1). There was increase in shoot iron concentration at 300 ppm Fe^{2+} in Ranjit followed by Siyal Sali. Mahsuri recorded lower shoot iron compared to other two varieties. Silveira et al.[8] reported the variation of rice shoots Fe concentration in his experiment. In that work, higher concentrations of Fe were detected in I409 plants, more susceptible to Fe toxicity. Shoot concentrations detected were up to 2.5X higher than in E108 plants (tolerant to Fe toxicity). The authors suggested, therefore, that E108 plants were more resistant to excess Fe due to the possible induction of avoidance mechanisms, allowing the plant to decrease Fe translocation to shoots.

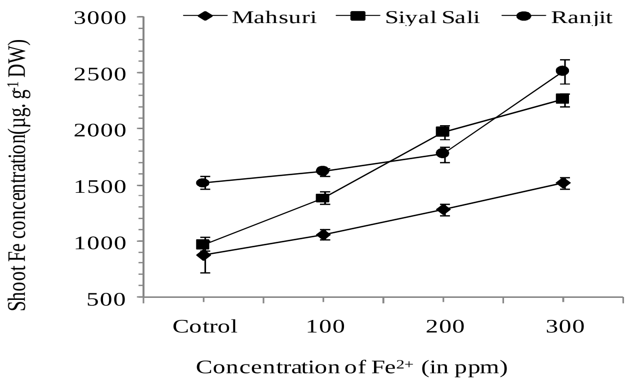

Figure 1: Effect of Different Levels of Fe^{2+} on Shoot Fe. The Vertical Bars Represents the Standard Error

In the present investigation a significant reduction (F value at 0.1% level of probability) of shoot Fe concentration predicted in Mahsuri might be due to induction of exclusion and/or avoidance mechanism in the roots and also due to formation of ferrihydrite layer on the root surface, most of this Fe seems to not be translocated to shoots[11,28,29].

Yield and Yield Attributing Parameters

Considerable variations in days to flowering and days to maturity have been observed in the varieties Ranjit and

Siyal Sali (Table 1) at 300 ppm Fe^{2+}, indicating the impact of higher iron on developmental phases in the life cycle of these varieties. Similar effects were not observed in the variety Mahsuri which exhibited luxuriant vegetative growth at this concentration of Fe^{2+}.

Table 1: Effect of Fe^{2+} on Phenological Phases of the Plants

Varieties↓	Treatments↓	Phenological phases		
		Days to panicle initiation	Days to flowering	Days to harvesting
Mahsuri	control	88	92	147
	100 ppm Fe^{2+}	88	93	146
	200 ppm Fe^{2+}	89	93	146
	300 ppm Fe^{2+}	88	93	147
Siyal Sali	control	90	103	160
	100 ppm Fe^{2+}	91	106	160
	200 ppm Fe^{2+}	95	108	166
	300 ppm Fe^{2+}	95	108	169
Ranjit	control	90	104	159
	100 ppm Fe^{2+}	95	104	159
	200 ppm Fe^{2+}	98	109	166
	300 ppm Fe^{2+}	98	112	169

At an exposure to 300 ppm Fe^{2+} iron, filled grain (%), 1000 grain weight and grain yield per plant decreased in Ranjit and Siyal Sali (Table 2 & 3). The stable grain weight in the form of 1000 grain weight and grain filling efficiency in the form of high density grain (94.67%) was observed in Mahsuri at 300 ppm Fe^{2+} (Table 3). Decrease in grain yield in Ranjit and Siyal Sali might be due to inefficient grain filling at higher Fe^{2+} in the medium. Compared to control Fe^{2+} treatment, the effect of higher Fe^{2+} in the growth medium is less prominent on yield and yield attributing parameters of a tolerant variety as reported by several research workers[3,9,10,30,31].

Table 2: Effect of Different Level of Fe^{2+} on Filled Grain (in %) and 1000 Grain Weight (in gm. $plant^{-1}$)

Impact of different levels of Fe (in ppm) on % of filled Grain						Impact of different levels of Fe (ppm) on 1000 grain wt.(gm. $plant^{-1}$)					
	Control	100	200	300	Mean		Control	100	200	300	Mean
Mahsuri	88	88	89.7	88.9	88.63	Mahsuri	19.97	19.17	19.1	19.47	19.425
Siyal Sali	78.3	69.3	48	40.3	59	Siyal Sali	24.5	22.67	19.6	16.3	20.77
Ranjit	69.3	61.3	48.7	37.3	54.17	Ranjit	19.57	18.4	17.3	15.5	17.7
Mean	78.56	72.89	62.11	55.51	67.27	Mean	21.34	20.08	18.68	17.1	19.3
SE(±M)	5.487	8	13.66	16.5	10.59	SE(±M)	1.66	1.2	0.66	1.2	0.88
Variables	F-value		CD at			Variables	F-value		CD at		
Replication	1.1 NS		5%	1%	0.10 %	Replication	0.79 NS		5%	1%	0.10%
Treatment	74.85***		3.05	4.15	5.58	Treatment	56.19***		0.715	0.973	1.31
Variety	321.99***		3.31	4.5	6.05	Variety	53.33***		0.619	0.843	1.134
T × V	22.17***		6.1	8.29	11.15	T × V	16.78***		1.239	1.683	2.264
CV (%)	5.36					CV (%)	3.79				

*** Significant at 0.1% level of probability

Table 3: Effect of Different Level of Fe^{2+} on High Density Grain (in %) and Grain Yield (gm. $plant^{-1}$)

Impact of different levels of Fe (in ppm) on % of HDG						Impact of different levels of Fe (in ppm) on grain weight (gm. $plant^{-1}$)					
	Control	100	200	300	Mean		Control	100	200	300	Mean
Mahsuri	94.67	93.67	95	94.67	94.5	Mahsuri	35.15	35.49	35.53	35.97	35.53
Siyal Sali	90.67	62.66	50.33	40	60.92	Siyal Sali	25.45	23.4	14.73	10.3	18.47
Ranjit	78.33	60.67	47	37.67	55.92	Ranjit	33.77	27	20.8	13.7	23.82
Mean	87.89	72.33	64.11	57.44	70.44	Mean	31.456	28.629	23.689	19.989	25.941
SE(±M)	4.8	10.68	15.5	18.5	12.25	SE(±M)	3.05	3.52	6.24	7.88	5.04
Variables	F-value		CD at			Variables	F-value		CD at		
Replication	0.98 NS		5%	1%	0.10 %	Replication	1.57NS		5%	1%	0.10%
Treatment	530.84***		1.67	2.27	3.06	Treatment	30.41***		2.714	3.689	4.964
Variety	1808.2***		1.46	1.98	2.67	Variety	118.58***		2.351	3.194	4.296
T × V	141.24***		2.89	3.93	5.29	T × V	9.10***		4.7	6.388	8.593
CV (%)	2.43					CV (%)	10.7				

***** Significant at 0.1% level of probability**

Grain Biochemical Components

Wide ranges of variations were observed in nutritional quality of grain among the cultivars. Marked reductions in crude protein content were recorded in the varieties Siyal Sali and Ranjit when subjected to higher external ferrous iron in the growth medium (Figure 2A). Of course the crude protein content was found to be stable in the variety Mahsuri even at 300 ppm Fe^{2+} concentration. Total soluble proteins in the grain were also found to be affected in the varieties Ranjit and Siyal Sali under higher Fe^{2+} concentrations. We observed a decreasing trend of grain soluble protein in the varieties Ranjit and Siyal Sali with the increment of Fe^{2+} concentrations (Figure 2B). Fe^{2+} ion induced oxidative damage in the plant tissues might be the reason of lower protein contents in these varieties. Grain soluble proteins remain unaltered in the variety Mahsuri indicating its better metabolic activities at higher iron concentrations. The iron tolerance capacity of this variety might be due to control translocation of soil iron to the shoots. Free amino acid (FAA) content of grain varied significantly among the cultivars (Figure 2C). Mahsuri recorded higher amino acid content compared to other varieties indicating its better adaptability to higher Fe^{2+} concentrations. In the present investigation we found that glutelin which is a major storage protein in rice endosperm, is the most dominant protein fraction followed by globulin and albumin (Figure 3). Among the varieties, Mahsuri recorded highest glutelin content than Ranjit and Siyal Sali signifying its better nutritional quality. Variations in protein fractions at Ranjit and Siyal Sali might be due to oxidative damage induced by toxic iron level. From these results of protein profiles, Mahsuri may be considered as the superior genotype among the three cultivars.

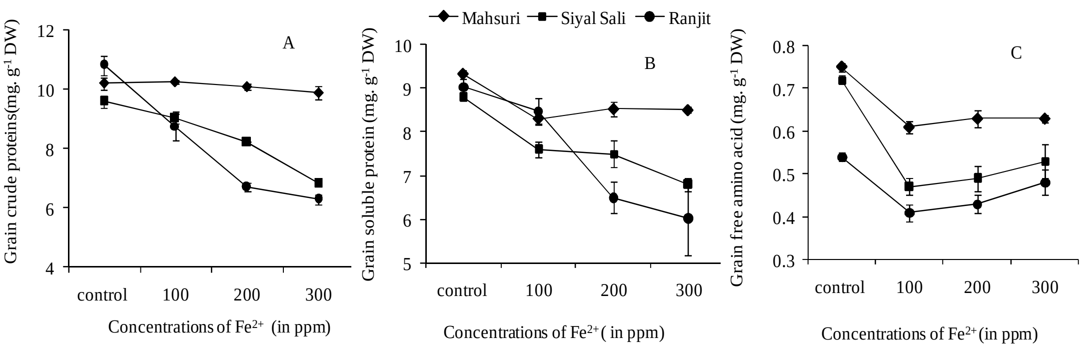

Figure 2: Affect of Different Levels of Fe^{2+} on Grain Crude Proteins (A), Soluble Proteins (B), and Free Amino Acid (C), The Vertical Bars Represent the Standard Errors

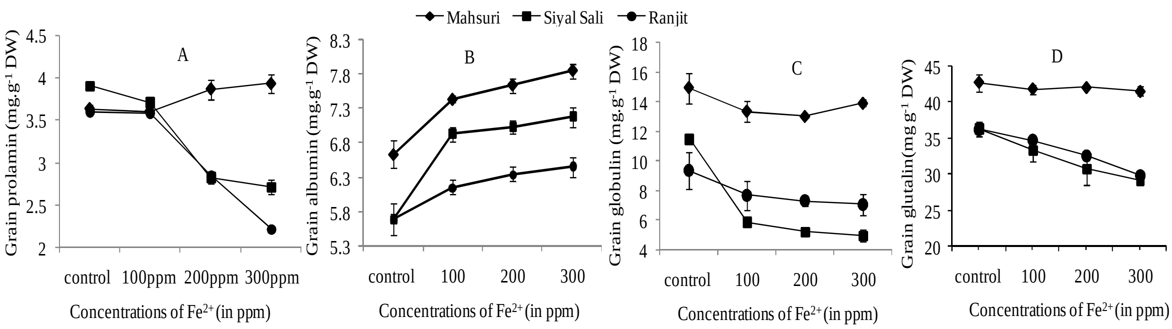

Figure 3: Effect of Different Levels of Fe^{2+} on Four Storage Proteins; (A) Prolamin, (B) Albumin, (C) Globulin and (D) Glutelin Content in Grain. The Vertical Bars Represent the Standard Errors

Results of total soluble sugars in the grain of the cultivars are presented in Figure 4 (A). Variety Ranjit recorded a decreasing trend of total soluble sugar (TSS) and reducible sugar (TRS) with the increment of Fe^{2+} concentrations while in

Siyal Sali 300 ppm iron was more effective in reducing TSS and TRS (Figure 4A, B). We did not observe any variations in TSS, TRS in the variety Mahsuri at different Fe^{2+} level.

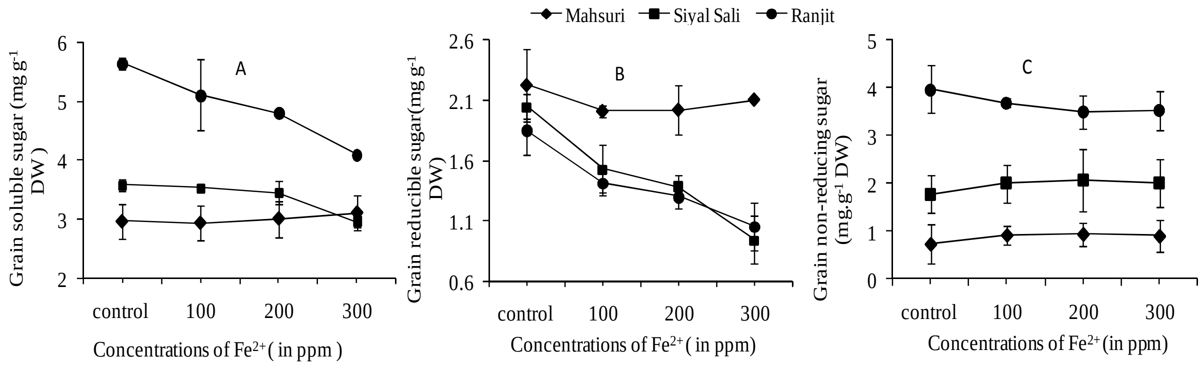

Figure 4: Effect of Different Levels of Fe^{2+} on Soluble Sugar (A), Reducible Sugar (B) and Non Reducible Sugar (C) in Grains. The Vertical Bars Represent the Standard Errors

In this experiment a considerable variations in the grain quality in terms of starch and amylose were observed among the cultivars (Figure 5A, B). These components were found to be stable at Mahsuri indicating its better response to higher Fe^{2+} in the soil. Due to severe growth damage of the plant during the developing phases, the varieties Ranjit and Siyal Sali could not maintain a steady level of biochemical components in the grains. Lower sugar content (Figure 4) and simultaneous carbohydrate mobilization to provide energy might be the reason of lower level of starch in Ranjit and Siyal Sali[32]. Highest fibre content in grain was observed in Mahsuri compared to other two varieties grown at different Fe^{2+} levels (Figure 6).

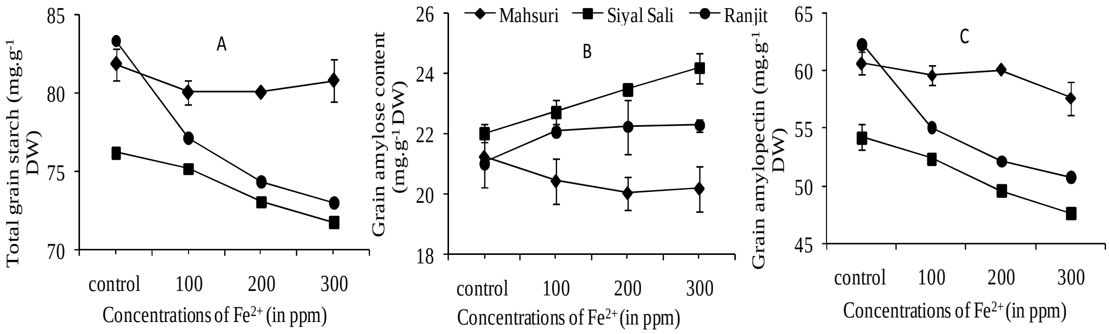

Figure 5: Effect of Different Levels of Fe^{2+} on Starch (A), Amylase (B) and Amylopectin (C) In Grains. The Vertical Bars Represent the Standard Errors

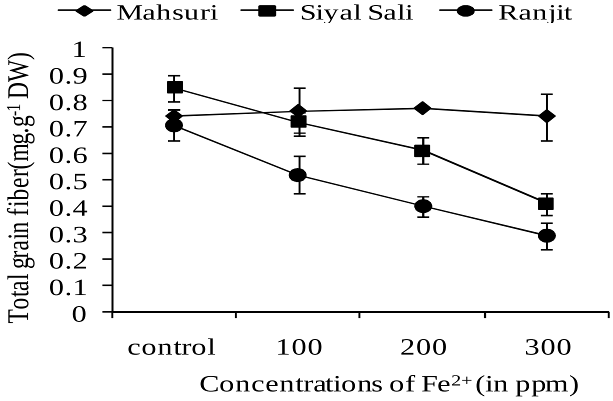

Figure 6: Effect of Different Levels of Fe^{2+} on Grain Fiber. The Vertical Bars Represent the Standard Errors

In present investigation, although a steady increase of grain Fe concentration was recorded in all the varieties with the increase of Fe^{2+} concentrations in the medium, Mahsuri is identified as better rice genotype in terms of highest grain iron concentration among the three cultivars (Figure 7A). No distinct impact of different levels of Fe^{2+} was observed on grain iron concentration of Mahsuri. Significant varietal differences were also observed in grain copper concentration (Figure 7B). Highest grain copper concentration was observed in the variety Mahsuri compared to Ranjit and Siyal Sali. The treatment effects were more pronounced in the varieties Ranjit and Siyal Sali. A decreasing trend of Cu concentrations was observed in these varieties at 200 and 300 ppm Fe^{2+} compared to Mahsuri. This variety also exhibited higher Zn at 200 and 300 ppm (Figure 7C). A gradual decrease in Zn concentration in the grains of Ranjit and Siyal Sali was clearly observed in our study. Although we did not observe any change in grains Mn concentration in Mahsuri, however, a decreasing trend of Mn concentration was seen at Ranjit and Siyal Sali when subjected to higher Fe^{2+} levels (Figure 7D).

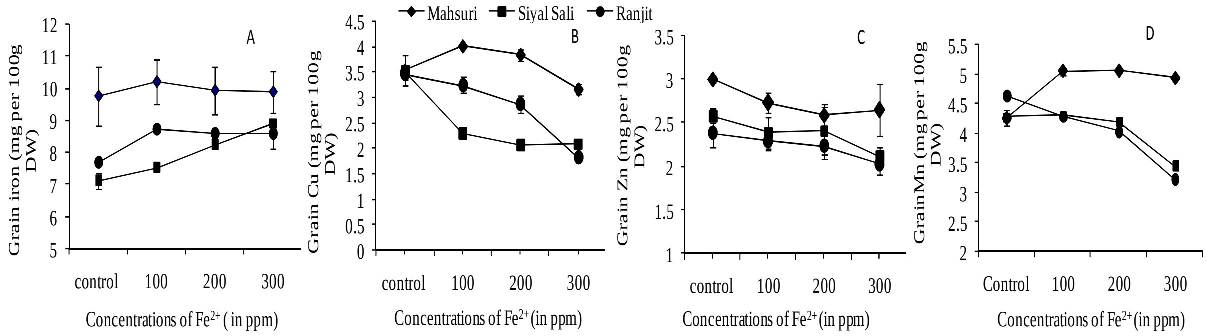

Figure 7: Effect of Different Levels of Fe^{2+} on (A) Fe, (B) Cu, (C) Zn and (D) Mn in Grains. The Vertical Bars Represent the Standard Errors

Mahsuri recorded a significant increase (F- value at 0.1% level of probability) in grain N and P, from control to 100 ppm Fe^{2+} in the medium and maintained a stable level at 200 and 300 ppm Fe^{2+} (Figure 8 A, C). N concentration in the grain of other varieties was significantly affected by higher iron in the medium. The lower nitrogen up-take by plants might be the reason of lower grain N and amino acid contents in these varieties. Figure 8(B) exhibits the variations in K concentration of the grains in all the rice varieties. Variety Mahsuri recorded a sharp increase in grain K level at 200 and 300 ppm Fe^{2+} while the potassium concentration decreased in other two varieties. Smaller K[7,14] and Cu[8,29] absorption rates under Fe-excess have already been reported in rice. It seems that, under Fe-excess, the relatively high ferrous iron concentration in the soil solution and uptake by the plant may result in static translocation of K and Cu to the grain of Ranjit and Siyal Sali.

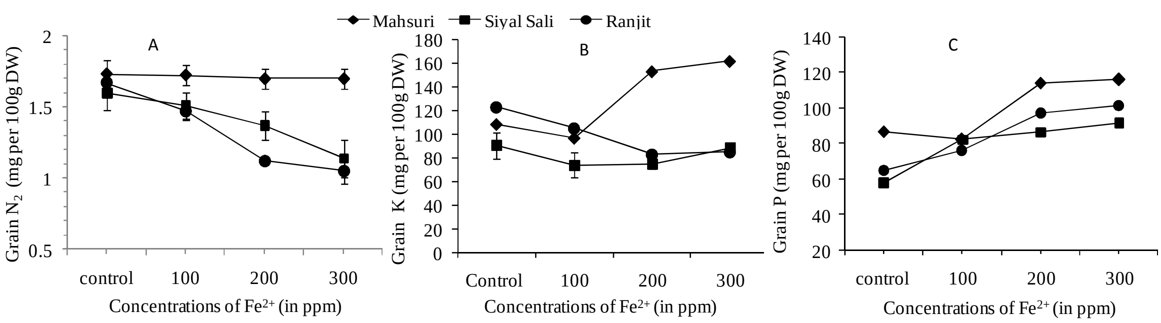

Figure 8: Effect of Different Levels of Fe^{2+} on Nitrogen (A), Potassium (B) and Phosphorous (C) In Grains. The Vertical Bars Represent the Standard Errors

Iron is an essential mineral for plants, required for biological red-ox systems[33] and vital component of many

enzymes that play important roles in the physiological and biological processes in plants. It involves in the synthesis of chlorophyll and is essential for the maintenance of chloroplast structure and functions. However, higher Fe^{2+} concentration in the soil may disrupt all physiological and biochemical processes in plants. Although plant develops sophisticated iron exclusion, inclusion and avoidance via internal compartmentation of Fe^{2+}, inclusion and tolerance via increased thresholds to elevated Fe^{2+} within leaf cells or ant-oxidative enzyme mechanism[34], under reducible soil condition, iron may be taken up in excessive quantities[35], which accelerate the formation of reactive oxygen species and also catalyses Fenton reaction generating toxic hydroxyl radicals in plants. These reactive species cause severe damage to root structure, chlorophyll, proteins and almost every organic constituent of living cells of iron sensitive cultivars leading to the plants death[1,29]. In the present investigation, the reduction in some biochemical components, grain soluble proteins, total soluble sugar along with amino acid accumulation in the grains of Ranjit and Siyal Sali at higher iron concentrations can be attributed to drastic growth reduction for oxidative stress, particularly impairment of ROS on plant cells, generated by iron toxicity. Several investigators have reported oxidative attack on proteins, fragmentation of proteins by ROS[36,37,14].

The variations of grain nutrients level in the rice cultivars might be due to their synergic or antagonistic interactions with increased iron concentration in the growth medium, a mechanism reported by other investigators[8,28,33]. In Mahsuri, selective and competitive complexation with organic chelators[38] and proper function of appropriate Fe transporters driven by suitable promoters[39,40,41] may be considered as the key in developing iron tolerance capacity with simultaneous accumulation of iron and other minerals in the grains. Varieties Ranjit and Siyal Sali, although recorded higher shoot iron at 300 ppm Fe^{2+} but grain iron level was lower than Mahsuri.

Plant Minerals may be remobilized from vegetative sources, but a major portion of minerals in seeds are likely to be supplied through continuous uptake and translocation during reproductive growth[11]. In Ranjit and Siyal Sali, shoot iron was not properly remobilized to seeds as is evident in case of Zn translocation to grain[41]. Moreover nutrients accumulate in the grain originate from uptake by roots after flowering to grain filling stages[11,29]. Thus lower rate of plant growth in terms of lesser tiller numbers and delayed flowering or inefficient expression of endosperm specific transporters might be another reason for lower grain iron concentrations in these two varieties. Since most minerals in grains would be obtained from soil after flowering, the plants from the susceptible genotypes which were already stressed and suffering from iron toxicity, performed worse in several aspects. Hence it would be reasonable to consider that their ability to take up minerals from the soil was also impaired by higher iron concentrations in the medium. We can proposed that in Ranjit and Siyal Sali less minerals could be absorbed by the plants, and therefore less minerals would be available to go to grain.

CONCLUSIONS

The varieties Ranjit and Siyal Sali execute poor micronutrient level and lower concentration of some biochemical components in grains possibly due to radical growth reduction and probable injury of ROS on plant cells at higher iron concentrations. Mahsuri, either due to limited Fe^{2+} uptake or exclusion of Fe^{2+} at root surface and its retention within the tissues with the expression of new proteins, maintained a stable micronutrient level in the grains upto 300 ppm Fe^{2+} and recorded better yield and grain quality components. The results indicate Mahsuri to be a suitable rice cultivar from growth and yield to quality characters for growing under iron toxic soil environment of eastern India. In conclusion, the findings confirm a negative impact of iron toxicity on the grains nutritional quality of iron sensitive rice genotypes, in contrast a genotype tolerant to iron toxicity being also able to maintain good grain nutritional quality.

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